9:45 a.m.

806-3 Treatment of Fulminant Myocarditis With Rabbit Antithymocyte Globulin: A Pilot Study

Timothy B. Icenogle, David Sandler, Ellen Klohe, Heart Institute of Spokane, Spokane, WA, International Heart Institute of Montana, Missoula, MT

Objectives: To evaluate possible efficacy of rabbit antithymocyte globulin (RATG) to treat biopsy proven myocarditis.
Background: In a murine model, antithymocyte serum improved survival in T-cell and humorally mediated viral myocarditis. RATG, an analogous drug used in human transplantation may have a role in treating myocarditis.
Methods: From Jan. 93 to Oct 02, all patients referred with biopsy proven myocarditis, (Dallas Criteria) were given RATG, either locally manufactured (200mg IM) or commercially prepared (Thymoglobuline 1.5 mg/kg IV), for a three-day course and then rebiopsied. Patients with active myocarditis on the repeat biopsy were given a second three-day course and rebiopsied. Adjunctive steroids were given in conjunction with RATG except in two patients on cardiac assist devices where steroids were contraindicated. Echocardiograms (echo) were obtained before RATG, at discharge and last follow-up.
Results: Six patients entered the study; all with marked reductions in ejection fraction (EF) by echo. Two patients did not require IV inotropes, two required IV inotropes and two required assist devices and inotropes. All patients had improvement in heart biopsies after RATG but one required a second course. One patient died of multiorgan failure while on an assist device. The five survivors had improvement in EF at the time of discharge over admission EF. Long-term follow-up, (mean 1466 days) showed the EF stayed the same or improved compared to the discharge EF in four patients but decreased in one.
Conclusion: RATG may be beneficial in the treatment of myocarditis. RATG causes a profound lymphopenia, inhibits cytokines, blocks co-stimulation molecules and may induce self-tolerance. A multicenter trial is needed to assess efficacy and safety.

10:00 a.m.

806-4 Cardiac Parvovirus Type B19 Infection Is Associated With Isolated Diastolic Dysfunction

Carsten Tschoepe, Mario Kasner, Matthias Pauschinger, Dirk Westermann, Wolfgang Poller, Uwe Kuehl, Heinz-Peter Schultheiss, Charite-Univ-Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany

Background: Cardiotropic viruses can influence diastolic LV function. In the present study, we report on the association of parvovirus B19 (PVB) genomes in the clinical setting of isolated LV diastolic dysfunction (LVDD).
Methods: 33 patients (18 women, 15 men; aged 47±13), presented with exertional dyspnoea, orthopnoea and/or reduced exercise tolerance and preserved systolic function (EF>55%) were included. LVDD was diagnosed when patients showed in addition abnormal transmitral inflow ratio (E/A) (<1, age-related), deceleration time (DT) (>240ms; age-related), end-diastolic pressure (EDP) (>16 mmHg), and/or wedge pressure (PC) at rest (>12 mmHg) and during exercise (>20 mmHg). We also investigated N-terminal (NT)-proB-type natriuretic peptide (BNP) plasma levels (Elecsys 2010, Roche Diagnostic, Germany) and endothelial dysfunction (ED) by intracoronary injections of ergonovine or acetylcholine. RV myocardial biopsy were taken for entero (EV) - and adenovirus (AV) genome analysis by nested PCR/RT PCR. Patients with coronary artery or valvular disease, LV hypertrophy, hypertension, or lung diseases were excluded.
Results: In 27 patients (82%) isolated LVDD was diagnosed. They differed significantly in all Doppler parameters, EDP, stress PC and in increased BNP levels (>10 pmol/l) ($P<0.05$) compared to patients without LVDD. In 24 out of 27 patients with LVDD ($P<0.01$) and in 2 of 6 patients without LVDD (n. s.) PVB19, AV and/or EV genomes were detected. With 92% PVB19 was the most frequent pathogen. ED was detected in 18 out of 26 examined patients. 16 patients with ED showed also LVDD. All of these patients were virus-positive ($P<0.01$). Only in 3 virus-positive patients with LVDD no ED was detected.
Conclusion: The genome of the vasculotropic PVB19 can be demonstrated in 89% of patients with isolated LVDD and is strongly associated with the incidence of ED. Thus, PVB19 induced ED-depend ischemia is a possible mechanism to induce LVDD.

10:15 a.m.

806-5 Short Interfering RNA Specifically Directed Against Protease 2A Lead to Significant Reduction of Coxsackievirus B3 Titers and Attenuation of Viral Cytopathogenicity

Marc Vorpahl, Christian Bradaric, Sabine Merl, Birgit Jaschke, Rainer Wessely, Deutsches Herzzentrum and 1. Med. Klinik, Technische Universitaet, Munich, Germany

Gene silencing by double stranded RNA molecules is a highly conserved, sequence-specific mechanism in eukaryotic cells. Coxsackievirus B3 (CVB3) is considered to be the most frequent cause of viral heart disease. At present, there is no specific antiviral drug available. Specific Aim: To design and evaluate a specific genome-based antiviral therapy directed against distinct CVB3 RNA regions encoding for essential non structural viral proteins. Methods: Several highly specific duplex siRNAs (21mers) were designed and synthesized against viral protease 2A, 3C or the viral polymerase 3D, respectively. A scrambled siRNA (SCR) served as a control. SiRNAs were transfected into Hela cells at a confluence of 40%. Cell survival following viral infection was analyzed by cell count of adherent cells and MTT inclusion, cytotoxicity by LDH ELISA. Viral replication was monitored by plaque forming assay. Results: There was no effect on cytopathogenicity and viral replication by all siRNAs directed against 3C or 3D. However, both siRNA-2A molecules led to a highly significant reduction of cytotoxicity 12h and 24h after infection (p.i.), when no cells from the 3C, 3D or SCR siRNA groups were adherent anymore. Accordingly, LDH release was significantly lower in siRNA-2A transfected cells up to 36h p.i.. Viral replication was significantly inhibited by over 100fold at the point of maximal viral replication in control cells (12h p.i.; 9.33E+07 vs. 1,1E+10 PFU/ml siRNA-2A vs. siRNA-SCR). Peak of viral replication siRNA-2A cells was not achieved until 48h p.i. at a 10fold lower absolute value. An additional control of siRNA-2A with exchange of 2 nucleotides had no effect on cell survival underscoring the specificity of our findings. The molecular pattern of CVB3 replication was altered significantly by siRNA-2A. Analysis of the therapeutic effect of siRNA-2A in vivo (mouse model) is currently investigated and will be presented. Conclusion: RNA interference against specifically against viral protease 2A leads to significant decrease of cytopathogenicity and viral replication in CVB3 infected human cells. This finding may have therapeutic implications since siRNAs may also be used for therapeutic approaches in vivo.

ORAL CONTRIBUTIONS

813 Cardiac Allograft Vasculopathy: Basic Mechanisms and Clinical Correlates

Monday, March 08, 2004, 11:00 a.m.-12:15 p.m.
Morial Convention Center, Room 217

11:00 a.m.

813-1 Cytomegalovirus Infectious Burden Is Proportional to Cardiac Allograft Vasculopathy in Heart Transplant Recipients

Luciano Potena, Carlo Magelli, Paolo Ortolani, William F. Fearon, Francesco Grigioni, Gaia Magnani, Fabio Coccolo, Alan C. Yeung, Helen I. Luikart, Sharon A. Hunt, Edward S. Mocarski, Jr., John P. Cooke, David B. Lewis, Angelo Branzi, Hannah A. Valantine, Stanford University, Stanford, CA, University of Bologna, Bologna, Italy

Background Cytomegalovirus (CMV) infection is a risk factor for cardiac allograft vasculopathy (CAV) in heart transplant (HT) recipients. However few human studies have addressed the question of infectious burden and its direct role in CAV severity. The aim of this analysis was to determine whether increasing viral burden was predictive of CAV.
Methods Forty-six consecutive HT recipients enrolled at Bologna (n=40) and Stanford (n=6) HT units (51±13 year; 78% males) were monitored for CMV activation, either by PCR or antigenemia testing of peripheral blood obtained weekly during the initial month, and then at monthly intervals after HT. CMV management consisted of either prophylaxis with ganciclovir for 4 weeks after HT, or pre-emptive treatment with ganciclovir only when antigenemia exceeded 50 cells/10^5 leukocytes. Patients were stratified into 3 groups based on viral burden: 1) CMV negative throughout follow-up (n=14); 2) asymptomatic positive PCR or antigenemia below 50 cells (n=16); 3) high CMV levels requiring treatment (n=16). CAV progression was quantified by coronary intravascular ultrasound (IVUS) performed at 1 and 12 months after HT
Results Overall, coronary intimal area (IA) increased by 70% ($P<0.01$) during the follow-up. IA increased by 30% in Group 1 recipients, by 90% in Group 2 and by 115% in Group 3 ($P=0.01$ for trend; $P<0.05$ for Group 1 vs. Group 3 and for Group 2 vs. Group 3, after correction for multiple comparison). This observation was confirmed analyzing change in intimal index ($P=0.03$ for trend among groups) and in maximal intimal thickness, which increased only in CMV infected patients ($P<0.05$). By multivariable analysis the degree of CMV infection remained an independent predictor of IA increase ($P=0.01$), even after correction for prophylactic vs. pre-emptive strategy, recipient's and donor's age, lipid blood levels and rejection score index.
Conclusion These data suggest that severity of CMV infection, as reflected by viral burden, is proportionally associated with the progressive increase in CAV, regardless of prophylactic vs. pre-emptive strategy. These results strengthen the hypothesis of a direct involvement of CMV in CAV pathophysiology.

11:15 a.m.

813-2 Incremental Value of Redox Pattern in Predicting Cardiac Allograft Vasculopathy After Heart Transplantation

Oberdan Parodi, Benedetta De Chiara, Fabio Turazza, Jonica Campolo, Andrea Garascia, Riccardo Bigi, Maria Frigerio, Silvio Klugmann, CNR Clinical Physiology Institute, Milan, Italy, Niguarda Ca' Granda Hospital, Milan, Italy

Background: Cardiac allograft vasculopathy (CAV) is the main factor limiting survival after heart transplantation (HTx). This study was aimed to identify predictors of CAV among clinical (age, cause of HTx, donor age, time from HTx, acute rejection and ischemia time), biochemical (fasting plasma glucose, creatinine, total cholesterol, LDL-cholesterol and triglycerides) and redox patterns [plasma total and reduced homocysteine, cysteine, cysteinylglycine, glutathione (GSH), reduced GSH in erythrocytes (GSHe) and vitamin E] variables.
Methods: 56 male patients (60±11 years) underwent angiography 53±36 months after HTx. Clinical (model 1), conventional biochemical (model 2) and redox pattern (model 3) data were sequentially entered into a multivariate logistic regression model to predict CAV. The increase in global χ^2 of the model after the addition of each block of variables identified an incremental diagnostic value.
Results: CAV was found in 18 (32%) patients. After adjusting for the most significant variables, GSHe (OR 0.99, 95% CI 0.987-0.999 per µmol/L, $p<0.02$) was independently associated to CAV. The addition of conventional biochemical variables reduced the over-